IDENTIFICATION OF ENVIRONMENTAL PREDICTORS OF DISEASE IN A WILDLIFE POPULATION

McKenzie J.[1], Morris R.[1], Pfeiffer D.[1]

Le possum Trichosurus vulpecula joue le rôle de réservoir de tuberculose à Mycobacterium bovis pour les bovins et les cerfs dans certaines régions de Nouvelle-Zélande. Les programmes de contrôle des possums dans ces régions coûtent cher. Ici une enquête cas-témoin a été réalisée pour chercher les facteurs d'environnement capables de prédire la localisation de cas de tuberculose chez les possums. Les paramètres étaient comparés entre zone à possums non tuberculeux et zones à possums tuberculeux. Les paramètres représentaient le couvert végétal, les espèces végétales, les refuges, le relief. Deux modèles de régression logistique ont été utilisés pour faire des prévisions. La pente du terrain et la présence de nombreux gîtes bien protégés sont associés aux cas multiples de tuberculose (p<0,05). La pente du terrain, la hauteur moyenne des arbres et l'abondance de végétation près du sol sont associés (p<0,05) à des cas isolés de tuberculose. Le but est de construire un système d'information géographique (SIG) pour prédire les zones à risque et d'y concentrer les moyens de lutte.

INTRODUCTION

Tuberculosis (TB) due to infection with Mycobacterium bovis is endemic in brushtail possums (Trichosurus vulpecula) in some areas of New Zealand. Possums in these areas are acting as a reservoir of infection for farmed cattle and deer which is hampering the eradication of TB from these farmed species. Current methods of controlling possum populations successfully reduce the prevalence of TB in possums and in-contact cattle and deer populations. However, eradicating TB from possum populations is difficult, and costly on-going programs are required to maintain possum populations at a low level to limit the spread of TB from possums to cattle and deer. New strategies are required to produce results that are more economically sustainable in the long term.

TB in possums is clustered both spatially and temporally (Pfeiffer, 1994), and some clusters of TB have been known to remain at the same location for many years (Hickling, 1992). Field data on the spatial distribution of TB in possums indicates that spatial clusters fall into two categories: endemic and sporadic. An endemic cluster is where TB is firmly established in the possum population and possum to possum transmission is occurring at the site. Such a site is commonly referred to as a "hot spot". A sporadic cluster is where there is little spread of TB amongst the possum population at the site and the cluster may disappear temporarily or permanently. The spatial scale of an endemic cluster is estimated to be in the order of 50 meters. The ability to predict the location of clusters of infected possums, and the ability to differentiate sporadic from endemic clusters would enable targeting of control measures which could increase the probability of eradicating TB from possums. This paper describes the approach used to determine if environmental predictors of clusters of TB in possums could be identified and to determine if these predictors could be used to differentiate endemic and sporadic clusters of TB.

METHODS

A case control approach was used to study environmental factors associated with possum habitat. The unit of interest was a 50 square meter area surrounding a possum trap site. Trap sites were selected from areas around the country where TB surveys of the possum population had been conducted and the location of TB-positive and negative possums had either been recorded or could be recalled to an accuracy of 20-30 meters. A TB-positive trap site was defined as one where one or more of the possums trapped at the site had gross TB lesions on post mortem examination. TB-positive trap sites were further subdivided into single and multiple TB sites. A single TB site was one where only one TB-positive possum had been caught. A multiple TB site was where more than one TB-positive possum had been caught. A TB-negative trap site was one where no TB-positive possums had been caught at any time. Negative sites were at least 100 meters from the nearest boundary of a TB-positive site. One TB-negative site was selected for every positive site from within the same survey area, generally on the same farm or a neighbouring farm. This method was adopted to increase the chance that possums at negative sites had the same risk of being exposed to TB as those at positive sites. Negative sites were randomly selected from all eligible sites within the survey area. A total of 134 sites was included in the study, comprising 68 TB-positive sites and 66 TB-negative sites. The TB-positive sites comprised 27 multiple and 41 single TB sites.

Data were collected on three sets of environmental factors: vegetation, den (nest) and topographic factors. Vegetation factors related to the abundance of each plant species within five different height tiers (>12, 6-12, 3-5, 0.3-2, <0.3 meters), mean height of the tallest trees at the site and presence or absence of trees with a circumference at breast height (cbh) greater than 75cm. Abundance of plant species represented the proportion of the site covered by each species if they were projected down to ground level, and was scored using the Braun-Blanquet scale (Kent and Coker,1992) with six categories (<1, 1-5, 6-25, 26-50, 51-75, 76-100%). The proportion of the ground covered by vascular vegetation, moss, water, large logs and roots, and bare was also recorded.

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[1] Department of Veterinary Clinical Sciences, Massey University, Palmerston North, New Zealand

Abundance of epiphytes was recorded on a subjective scale of 1-5. Vegetation data were collected using the Recce inventory method (Allen,1992) which was designed by the New Zealand Forest Research Institute for rapid broad-scale surveys of compositional variation in mountainland forests.

Den factors related to the location, abundance and quality of available possum den sites. Quality of a den site was scored on a scale of 1 to 4 depending on the degree to which the den was exposed to the environmental elements, with one being completely exposed and 4 being completely enclosed. The abundance of dens of each quality score at each location was scored on a subjective scale of 1-5. Topographic features included the mean slope and aspect of each site.

Univariate analyses were conducted to screen out individual variables that were not associated with TB status of the sites. Chi-squared analysis was used for categorical variables and the Mann-Whitney U test for difference between medians was used for continuous variables as none of these was normally distributed. Variables that were significantly associated ($p < 0.2$) with TB status of the sites were then used in multivariable analyses.

Multiple correspondence analysis was used to explore the relationships both between the independent variables themselves and between the independent variables and the three TB status variables which were entered as dummy variables. Continuous variables were categorised so they could be included in the multiple correspondence analysis.

Two unconditional logistic regression models were produced: one to predict the probability that a location was a multiple TB site rather than a negative TB site, and the other to predict the probability that a location was a single TB site rather than a negative TB site using the set of environmental factors that were selected from the univariate analyses. A forward selection process was used with a cut-off p-value of 0.05. Interaction between the variables that were selected was tested using the likelihood ratio statistic. The continuous variables such as slope, height and percent abundance within height tiers were categorised using quartiles to produce units of measurement which are easier to interpret. A receiver operating characteristic (ROC) curve was generated for each of the two regression models. It enables the identification of an appropriate cut-off point, with a particular sensitivity and specificity, to classify sites as multiple or negative, or as single or negative.

The statistical analyses were conducted in NCSS version 6.0.2 (NCSS, Kaysville, Utah 84037, USA). Multiple correspondence analysis was conducted in Statistica (StatSoft Inc., Tulsa OK 74104, USA).

Table I
Regression coefficients expressed as odds ratios with their associated p-values for the two logistic regression models

Variable	Multiple vs negative TB	Single vs negative TB	Description
Slope	0.3 (.000)	0.5 (.003)	Slope of the land, divided into four 13° categories
Mult den4	4.1 (.01)		Presence/absence of multiple dens of quality 4
Height		2.2 (.01)	Mean top height of trees, divided into four 9-meter categories
T5 cover		81 (.10)	Percent of tier 5 (0.3-2 meters) covered with vegetation, divided into four categories
Ground veg		40 (.01)	Abundance of vascular vegetation at ground level divided into four categories
Veg*T5cov		0.07 (.06)	Interaction between vegetation and abundance of cover in tier 5

RESULTS

Variables that were put forward for multivariable analyses were: slope, mean top tree height, presence/absence of trees with cbh >75cm, percent vegetation cover in tiers 4 and 5, percent ground covered in vascular vegetation, percent ground covered with large logs and roots, total abundance of good den trees, total abundance of poor den trees, abundance of dens of each quality score:1, 2 and 3, and the presence/absence of multiple dens of quality score 4. Multiple correspondence analysis using this group of independent variables showed a greater differentiation between multiple TB sites and both single and negative TB sites. There was considerably less differentiation between single and negative TB sites.

Results from the two regression models are presented in table I. ROC curves for the two models used to predict TB status of a site are shown in figure 1. A cut-off probability of 0.33 for multiple TB prediction and 0.32 for single TB prediction maximises sensitivity and specificity of each model respectively.

Figure 1
ROC curves of two multiple logistic regression models to predict the TB status of possums in different locations using environmental factors

ROC curve of multiple versus negative TB sites   ROC curve of single versus negative TB sites
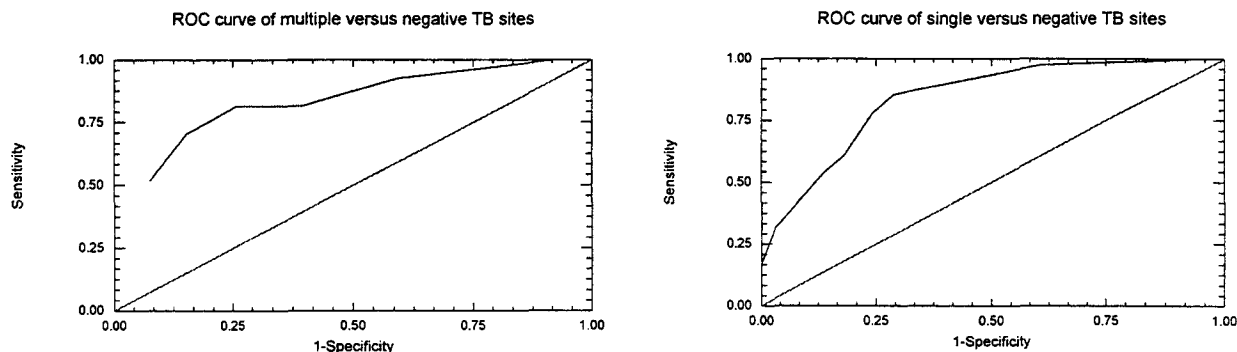
DISCUSSION

The results of this study indicate that TB possums are more likely to be found on flat or gently sloping land covered with tall trees with extensive vegetation either at ground level or at the 0.3-2 meter level, but without dense vegetation extending from a height of 2 meters to ground level. Areas with highly enclosed den sites, such as in hollow logs or under large root systems, are more likely to be endemic TB sites or "hot spots". These results add further evidence to support the concept of sporadic and endemic clusters of TB occurring in possum populations. Endemic clusters are thought to be present over a long term period and are associated with areas where TB continues to spread amongst possums inhabiting the area. Research on the epidemiology of TB in possums suggests that the major spread of TB between possums occurs in the vicinity of dens. This is supported by the significance of the den-related factor to differentiate multiple from negative TB sites. Highly enclosed dens provide an environment which favours the spread of TB as possums are more likely to occupy a shared air space in the denning area. The absence of den related factors in differentiating single from negative sites would suggest that these are more likely to be sporadic sites where TB does not spread so readily between possums and thus does not become established at the site.

The study identified environmental factors that can be used to predict the location of both endemic (multiple TB) and sporadic (single TB) clusters of TB in possums. Information on these factors will be used in a geographic information system to generate maps of possum TB risk for areas of habitat, and identify potential TB possum "hot spots". The availability of such a prediction system will help with the development of TB management strategies both at an individual farm level and at a wider district level, and it offers the opportunity to focus control measures more tightly in areas where they produce the greatest effect.

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